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Shedding light on shedders

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ABSTRACT

All previous examinations of the shedder status of individuals have been based on conclusions inferred from the amount of DNA deposited by donors after they have held an object for a fixed period of time. In all interpretations of shedder status experiments have involved a range uncertainties, especially in regards to results arising from studies carried out in different laboratories. These apply to the efficiency of the swab collecting DNA from the item touched, the amount of DNA left on the swab after attempts to recover it, and the percentage loss of DNA during the lysis and extraction processes. No previous study has attempted to mitigate these uncertainties or verify how much of the DNA deposited was collected through swabbing, how much DNA present on the swab was recovered or how much DNA is lost during the extraction process.

We present a study that accurately measures the deposition, collection and amplification of DNA deposited by a range of donors allowing for an accurate determination of the shedder status of individuals. Eleven donors were asked to wash their hands and then deposit a thumbprint onto glass slides by making pressure for 15 seconds 0, 15, 60 and 180 minutes after handwashing. Both left and right thumbs were used and all testing was performed in triplicate. Measurement of the quantity of cellular material deposited on the slides was carried out using DiamondTM Nucleic Acid Dye and fluorescence microscopy on each of 264 thumbprints. Fluorescence microscopy was then used to demonstrate that all the DNA present on the slides was recovered by the swabbing operations and then direct PCR, using the Identifiler™ Plus kit, was used to ensure that none of the DNA present on swabs was lost during DNA profiling.

The combination of using a DNA binding dye and direct PCR allowed an accurate means of measuring the extent to which individuals exhibit different extents of shedding. This small study, 11 donors, showed that individuals fell into one of three distinct groups: heavy, intermediate, and light shedders, regardless of the hand used.

1. Introduction

There continues to be much research into the transfer of DNA. Central to the means by which DNA transfers during direct contact between skin and a substrate is the shedder status of the person touching an object. The concept of a shedder or non-shedder came from the now well-cited paper by Lowe et al. [1]. By holding plastic tubes at a controlled time after handwashing, it was found that some donors had a higher propensity to transfer their DNA than other donors; and so came about the concept of a 'shedder' or 'non-shedder'. There ensued a number of follow-up examinations with initially varying results [2,3] but comprehensive studies have more recently shown that there is a difference between peoples' propensity to transfer DNA, regardless of gender or hand-dominance [4–7].

In all previous studies, donors were asked to hold or make direct contact with an object for a defined period of time. This was either after known time intervals post-handwashing or without this knowledge to simulate real-world scenarios [8]. Following the direct contact, an area was sampled with a swab and the process of DNA extraction performed. The method of DNA extraction varied from Chelex® to automated liquid handling systems using either solid phase extraction or magnetic beads. Based on the amount of DNA recovered, and any subsequent short tandem repeat (STR) DNA profile, a conclusion could be made as to the shedder status of the donor. All previous studies started by collecting transferred biological material to a swab, and as any swabbing process is unlikely to collect 100% of the cellular material deposited on contact this process needs to be uniform in collection and the same relative amount of cellular material was removed from every sample collected. During the DNA extraction process the same relative amount of the DNA on the swab needs to be released into the initial buffer used for the DNA extraction process and that the amount of DNA isolated during the extraction process is uniform for all samples tested and that the percentage loss of DNA during this DNA extraction process is the same for trace amounts of DNA compared to large amounts of initial starting

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DNA.

We present a study where the amount of DNA transferred by contact at specific time intervals post-handwashing, and through subsequent collection using a swab, can be estimated and recorded as a series of images collected using fluorescence microscopy. DNA can be detected by Diamond™ Nucleic Acid Dye as this is a molecule that binds to an external groove in DNA. Importantly, Diamond™ Nucleic Acid Dye cannot bind effectively to microbial DNA and it has recently been shown to detect DNA with no subsequent effect on the amplification process [9,10]. The dye has no known mutagenic effect at the concentrations used, is very inexpensive, and as it has an excitation of 494 nm and emission at 558 nm such that it can be visualised using a fluorescence microscope with a filter at 510 nm even under ambient lighting. Diamond™ Nucleic Acid Dye has only recently been applied to hair, saliva, skin flake [10,11] and here it is used specifically to determine shedder status.

Direct PCR gained much prominence when first shown to generate DNA profiles from biological deposits on fibres [12], and has more recently through its use to effectively amplify DNA from fingermarks [13–15], single hairs [16] nails [17] and items of forensic interest [18]. This current study combines the use of a DNA staining dye with direct PCR to examine the shedder status of volunteers in a simple and easy to perform method. The study was designed to show whether all the DNA transferred at the point of contact was collected by the swabbing method with this process viewed in real-time. Further, by using direct PCR we circumvent the issue regarding unknown loss during the DNA extraction process and can make a direct correlation with the amount of DNA that was collected on the swab and the subsequent DNA profile.

2. Methods

Approval from the Social and Behavioural Research Committee (reference 7569) was obtained prior to initiating this project.

2.1. Deposition of DNA

A total of 11 individuals comprising 5 males (designated M1 – M5) and 6 females (designated F1 – F6) washed their hands using water. Glass slides were used for contact. These were cleaned with 3% bleach, followed by wiping with absolute ethanol, and were then irradiated with ultraviolet (UV) light, by being placed approximately 3 cm below from UV lamp for 15 min, before use to ensure no DNA was present. Control samples were collected from areas of the glass prior to any deposition of a thumbprint. The volunteers made contact with these clean DNA-free glass slides with their left and right thumb for 15 s with medium pressure. The time intervals post-handwashing were: 0 min, 15 min, 60 min and 180 min. All tests were performed in triplicate creating 264 data-sets.

2.2. Staining of DNA

The slides were stained with 20 fold dilutions of the stock (10,000 x) solution of Diamond™ Nucleic Acid Dye (Promega, Madison, WI, USA). A Dino-Lite fluorescent digital microscope (AnMo Electronics Corporation, New Taipei City, Taiwan) equipped with an emission of filter of 510 nm and a blue LED excitation light source (480 nm) was used to visualise the presence of dyed material, referred to as cellular material hereafter. Scoring of cellular material abundance was performed by counting the number of bright spots in three frames (each 1 mm^2) under the microscope at 220 × magnification.

2.3. Collection of DNA

A micro-applicator (ultra-fine) swab (City Dental, Adelaide, Australia) was used to collect material from the slides. The swabs were moistened with 2 µL of 0.1% Triton-X (Sigma, Victoria, Australia) solution and applied to the entire thumbprint on each slide.

2.4. Amplification of DNA

Direct PCR was performed using the Identifiler Plus™ kit (Thermo Fisher Scientific, Melbourne, Australia) by removing the swab head directly into a 0.2 mL thin-walled PCR tube. Amplification was performed in 25 µL, with exceptions to the validated protocol being 2 µL of Prep-*n*-Go™ (Thermo Fisher Scientific, Melbourne, Australia) and Low TE Buffer (Thermo Fisher Scientific, Melbourne, Australia) in place of water. All amplifications were performed using a ProFlex thermal-cycler (Thermo Fisher Scientific). The total number of cycles was the validated 29 cycles. PCR product (1 µL) was added to 8.7 µL Hi-Di formamide and 0.3 µL 600 LIZ™ (Thermo Fisher Scientific, Melbourne, Australia) and separated on a 3500 Genetic Analyser (Thermo Fisher Scientific, Melbourne, Australia). Data were analysed using GeneMapper ID-X (version 1.4).

3. Results & discussion

An image of the fingermark deposited on a glass slide and stained with Diamond™ Dye is shown in Fig. 1A. An area not touched by the donors, but stained with Diamond™ Dye, is shown in Figure S1 to illustrate that no background fluorescence was recorded from the glass substrate. This allowed the verification that all the cellular material visualised within the fingermark samples were deposited by the donor. As indicated previously, Diamond Dye cannot bind effectively with microbial DNA therefore it can reasonably be assumed that the dots represent the location of human DNA deposits on the slides. The dye used binds with cell-free DNA as well as DNA within a nucleus, however, it is possible that dyed, cell-free DNA would be too small to observe as discrete spots using fluorescence microscopy and may contribute to the background fluorescence. Diamond™ dye does not bind efficiently to single-stranded mRNA therefore although there may be some fluorescence from mRNA, therefore it has been assumed that the majority of the material visualised with the dye is from cellular DNA. It has been reported that cell-free DNA is deposited by touch [19] and both this cell-free DNA and DNA within nuclei will be deposited for potential collection. Fig. 1B shows a swab in the act of removing cellular material. A 'clean' area on the slide is visible where cellular material has been removed from the slide and transferred onto the swab. Fig. 1C shows the same area after swabbing the entire area to confirm that all the stained cellular material that was deposited had been effectively removed. Fig. 1D shows the same swab with stained material now placed directly into the PCR tube, confirming that the cellular material collected was now a template for direct PCR. A sterile swab and a sterile swab stained with Diamond™ Dye were placed into PCR tubes and visualised, as shown in Figure S2, to demonstrate the lack of background fluorescence when compared to the swab that collected cellular material.

The amount of cellular material deposited on each glass slide was recorded, in triplicate, for each of the 11 volunteers' thumbprints at the four time points (time point 0, 15 min, 60 min and 180 min post-handwashing). These data are shown in Fig. 2. It is evident that between time point 0 and 15 min there is a large increase in the amount of cellular material available for deposition within the thumbprint. The accumulation plateaus by 1 h post-handwashing.

The 11 donors appear to fall into one of three categories, as evident in Fig. 2. Two male donors fall into the heavy shedder status. Four female donors fall into the light shedder status. The remaining 5 donors fall into an intermediate shedder status and comprise 3 males and 2 females. These three categories have been proposed previously [20] and although supported by this study there is evidence of a gradation of shedder status from heavy to light.

Direct PCR was performed on samples taken 60 min post-handwashing using the Identifiler Plus™ kit. This amplifies 15 STR loci plus